Biofilm system 1

Development and architecture of *E. coli* biofilms

Introduction

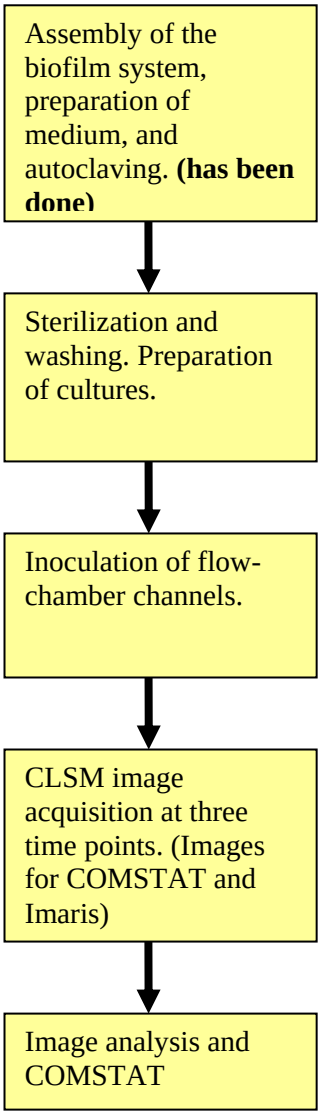
In a investigation, Ghigo (2001) presented evidence for an inverse relationship between plasmid conjugation and biofilms, from which he concluded that the presence of actively conjugating plasmids in *E. coli* cells induce biofilm development, and this stimulatory activity was coupled to the synthesis of conjugation pili attached to the bacterial cell surface. This could mean that plasmid transfer may be an auto-stimulatory process, which is initiated in cases where bacterial cells are close to each other (often the case on surfaces), and which subsequently induces biofilm development resulting in improved conditions for plasmid transfer.

Aims

In the present exercise you are going to compare *E. coli* and different *tra* mutants ability to form biofilms in flow chambers. You will take images at 3 different time points during growth and afterwards quantify differences between the mutants using a computer program, COMSTAT.

Reference:

Reisner A, Haagensen JA, Schembri MA, Zechner EL, Molin S. Development and maturation of Escherichia coli K-12 biofilms. Mol Microbiol. 2003 May;48(4):933-46.


Assembly of the biofilm system, preparation of medium, and autoclaving. **(has been done)**
Sterilization and washing. Preparation of cultures.
Inoculation of flow-chamber channels.
CLSM image acquisition at three time points. (Images for COMSTAT and Imaris)
Image analysis and COMSTAT

Experimental procedures

Day 1. Sterilization and washing of the biofilm system, saturate tubings with media over night.

See the protocol for biofilm work.

Preparation of cultures.

4 different strains are going to be investigated, each team will have one set of the following strains/each team has 4 channels. Data from all teams will be pulled in the end for quantification:

***E. coli* haboring the F plasmid (F+)**

***E. coli tra* mutant (F*traA*), no pilus synthesis**

***E. coli tra* mutant (F*traD*), no DNA transfer**

***E. coli tra* mutant (F*traQ*), no pilus processing**

The strains should be inoculated in 10mL LB and incubated O.N. at 30°C.

Day 2, morning. Inoculation of flow-chamber channels.

See the protocol for biofilm work

The O.N. cultures should be diluted to OD_{450}=0.05 in 0.9% NaCl before inoculation.

Day 2 afternoon. CLSM image acquisition.

All microscopic observations are performed by the use of a Zeiss LSM510 Confocal Laser Scanning Microscope (CLSM) equipped with lasers, filter sets, and detectors for monitoring of Gfp (excitation 488nm, emission 517nm). The images are acquired with a 40x/1.3 Plan-Neofluar oil immersion objective.

Day 3, morning and afternoon. CLSM image acquisition, cont.

Image analysis.

Prepare images of the different biofilms by the use of the Imaris software and quantify differences or similarities between wild type and *tra* mutants using COMSTAT.

Strain list (see Reisner et al):
SAR18-1: *E. coli* wild type (no *F'*), *gfp+*
SM2028: *E. coli*, pOX38:*F+*, *gfp+*
SM2029: *E. coli* pOX38:*FtraD, gfp+*